

PHENOLIC CONTENT DISTRIBUTION AND ANTIOXIDANT ACTIVITIES OF *TERMINALIA SERICEA* BURCHChinedu P. Anokwuru^{1*}, Isaiah D.I Ramaite¹, Pascal Bessong²

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Abstract

Background: *Terminalia sericea* has been used traditionally for the treatment of diseases associated with oxidative stress. This study was aimed at determining the distribution of phenols in the leaves, stem bark and root bark of *Terminalia sericea* and their antioxidant activity.

Materials and methods: Hot and cold water, methanol/acetone extracts were evaluated for their total phenolic content (TPC), flavone/flavonol content (FFC), flavonone/dihydroflavone content (FDFC), hydroxycinnamic acid derivative content (HCAC) and tannin content (TC). DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals and reducing power assays were used to assess the antioxidant activity.

Results: The leaves had the highest average TPC (440) expressed in milligram Gallic Acid Equivalent (mgCAE) /gram of the extract. The leaves also had the highest average TC (7.14) expressed in milligram Quercetin Equivalent (mgQE) /gram of the extract. The stem had the highest average FDFC (19.23 mgQE/g) while the root had the highest average FFC (74.76 mgQE/g) and HCAC (214.57) expressed in milligram Caffeic Acid Equivalent mgCAE/ gram of the extract. The stem exhibited the highest average DPPH free radical scavenging (9.85 µg/mL) and reducing power (6.01 µg/mL) activities. Water was a better extracting solvent for TPC and FDFC while methanol/acetone was a better extracting solvent for FFC and HCAC. The correlation between TC and reducing power activity ($r=0.668$) at $P < 0.05$ suggests that tannins were responsible for the antioxidant activity.

Conclusion: This study has shown that the distribution of phenolics differs in the organs of *T. sericea*, and could affect the quality of medicinal products sold.

Key words: Phenolic contents; *Terminalia sericea*; Antioxidant activity; Distribution studies.

Introduction

Polyphenols are widely distributed secondary metabolites of plants with approximately 8,000 compound structures reported. They vary greatly between different species, and cultures, and with maturity, season, region and yield. They are classified according to their structures as phenolic acid derivatives, flavonoids and tannins (Alberto et al., 2006; Zwenger and Basu, 2008). Natural phenolic compounds are found in leaves, fruits, bark and wood and can accumulate in large amounts in particular organs or tissues of the plant (Nitiema et al., 2012). Phenolic compounds present multiple biological properties which are of growing interest due to the high antioxidant, anti-proliferative, anti-inflammatory, anti-allergic, antithrombosis, antitumor, antimicrobial, anti-triglyceride deposit, anticholesterolemic and antiviral activities (Alberto et al., 2006; Sandhar et al., 2011). The molecular basis for the chemical and biological effects of phenolics is attributed to the ability to readily donate electrons and protons in sequence. They protect plants from oxidative stress through their antioxidant activity while their potential toxicity and harmful effect on herbivores and pathogens result from their prooxidant activity (Tuominen, 2013a).

Terminalia sericea Burch. Ex. DC (Combretaceae) is an abundant plant in the tropical and warm temperate regions, especially in Africa. Ethnomedical information revealed that this plant is commonly used for the treatment of cough, skin infections, diabetes, diarrhoea, and gonorrhoea (Eldeen et al., 2008). The dried fruit is used in a multicomponent recipe for the treatment of tuberculosis and the dried leaves are used for the treatment of dysentery. The water extract of the dried leaf is used to treat menorrhagia, while the powdered dried leaves are used to cover infected wounds. A decoction of the ground root is used for the treatment of bilharzia and stomach troubles. A decoction of the dried roots is used to prepare a soft porridge with maize flour for treatment of diarrhoea. A decoction of the plant is used in a multi-component preparation to enhance virility and for treatment of venereal diseases (Moshi and Mbawambo, 2005; Eldeen et al., 2008). It is also used for the treatment of hypertension and fever (Green et al., 2010). Anolignan B isolated from the ethyl acetate root extract was reported to possess antimicrobial properties (Eldeen et al., 2008) while lupeol and the inseparable set of mixtures of epicatechin-catechin and galocatechin-epigallocatechin are responsible for the antioxidant activity of the stem bark (Nkobole et al., 2011). All the organs of *T. sericea* have been reported to possess therapeutic properties but the root is the most used traditionally and scientifically studied. In our continuous search for biological active compounds especially from medicinal plants, we are concerned about preservation of these plants. Therefore, our study of the distribution of polyphenolics and antioxidants in the organs of *T. sericea* is the first step in finding out the best alternative organ for the root.

Materials and Methods

Plant Material

Fresh leaves, stem bark and roots of *T. sericea* were collected in May, 2013 from Muyexe village, Giyani, in the Limpopo region of South Africa. The plant was identified by Dr. P. Tshisikhawe of the Department of Botany, University of Venda and a voucher no. MT001 was

assigned and deposited in the university herbarium. The plant samples were rinsed with distilled water, cut into small pieces and air-dried at room temperature.

Extraction of Plant Material

The dried plant samples were grinded to a fine powder and stored in clean glass containers until further use. Five hundred (500) grams of the powdered plant materials were soaked in 1L of a methanol: acetone (MA) mixture (1:1) for 48 h with intermittent shaking at room temperature. The extracts were filtered and the residues re-extracted with the same volume of solvents for 24 h. All the filtrates of each extract were concentrated using a rotary evaporator at 40 °C. About 100 g of the root and stem bark powder, 50 g of leave powders were soaked in boiling water and cold water to obtain hot and cold water extracts respectively. The extracts were filtered after 24 h and evaporated. The concentrated extracts were finally dried in the oven at 60 °C. Working solutions (0.1mg/ml) of the extracts were prepared for the analysis.

Determination of Total Phenolic Content

This method was executed as described by Singleton and Rossi (1965). The assay is based on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds. The reduced Folin-Ciocalteu reagent is blue and thus detectable with a spectrophotometer at 760 nm. A one mL aliquot of each extract was added in a volumetric flask, containing 9 mL of water. One milliliter of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 mL of 7 % sodium carbonate was added to the mixture, and then incubated for 90 min at room temperature. After incubation, the absorbance against the reagent blank was determined at 760 nm. A reagent blank was prepared using distilled water instead of the plant extract. The amount of phenolic compounds in the extract was determined from the standard curve produced with varying concentrations (10, 20, 30, 40, 50 µg/mL) of gallic acid. All samples were analysed in triplicate. The final result was expressed as milligram Gallic acid equivalent per gram (mgGAE/g) of the extract.

Determination of Flavone and Flavonol Content

Flavone and flavonol content was quantified as described by Boulanonuar et al. (2013). Briefly, 0.5 mL of 2 % AlCl₃ in ethanol solution was added to 0.5 mL of each sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as standard for the construction of the calibration curve. All samples were analysed in triplicate. The final result was expressed as milligram Quercetin equivalent per gram (mgQE/g) of the extract.

Determination of Flavonone and Dihydroflavonol Content

The quantification of total flavone and dihydroflavonol contents was executed as described by Boulanonuar et al. (2013). Briefly, an aliquot (0.25 mL of sample or standard) and 0.5 mL DNP (2,4-dinitrophenylhydrazine) solution (0.25 g DNP in 0.5 mL 96 % sulphuric acid, diluted to 25 mL with methanol) were heated at 50 °C for 50 min. After cooling to room temperature, the mixture was diluted to 2.5 mL with 10 % (w/v) KOH in methanol. A sample (0.25 mL) of the resulting solution was added to 2.5 mL methanol and diluted to 12.5 mL with methanol. Absorbance was measured at 486 nm. The final result was expressed as milligram Quercetin equivalent per gram (mgQE/g) of the extract. Samples were analysed in triplicate.

Determination of Hydroxycinnamic Acid Derivatives Content

The hydroxycinnamic acid derivatives of the extracts were determined as described by Boulanonuar et al. (2013). Aqueous ethanol (95% v/v; 1 mL) containing 0.1% HCl was added to 1mL of each extract in a 10 mL volumetric flask, and the volume made up to 10 mL with 2% HCl. Absorbance was measured at 320 nm and the final result expressed as milligram Caffeic acid equivalent per gram (mgCAE/g) of the extract. Samples were analysed in triplicate.

Determination of Tannin Content

Contents of condensed tannins were determined by the procedure reported by Fellah et al. (2011). Hundred milliliters (0.1mg/ml) of the leaves, stem bark and roots extracts were mixed with 3 mL of 4% methanolic vanillin solution and 1.5 mL of 1N HCl. The mixture was allowed to stand for 15 min and absorbance measured at 500 nm. The final results were expressed as mg Quercetine equivalent per gram (mgQE/g) of the extract. Samples were analysed in triplicate.

Determination of DPPH Radical Scavenging Activity

Antioxidant activity was evaluated by determining the radical scavenging activity of each extract using a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) assay reported by Motamed and Naghibi (2010). Exactly 2 mL of 0.15 mM DPPH was added into 1 ml sample or standard solution of different concentrations and allowed to stand at room temperature for 30 min. A change in colour from deep violet to light yellow was observed due to the loss of hydrogen atom from reducing substances, giving rise to a more reduced form. After 30 min, the absorbance of the resulting mixture was measured with a UV-Visible spectrophotometer at 517 nm. The absorbance was converted into percentage antioxidant activity using the formula mentioned below. The IC₅₀ was calculated and the values represent the concentrations of the compounds that caused 50 % inhibition of radical formation.

Antioxidant activity percentage (AA %) by formula:

$$AA\% = (\text{Abs standard} - \text{Abs sample}) / (\text{Abs standard}) \times 100$$

Determination of Total Reducing Power

The reducing power was determined through the transformation of Fe^{3+} to Fe^{2+} induced by plant extracts according to the method reported by Ye et al. (2013). Sample solutions at different concentrations were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide solution (1% w/v). The mixture was incubated at 50 °C for 20 min in the oven. Afterwards 2.5 mL TCA (10%) were added and the mixture was centrifuged at 2500 rpm for 10 min. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL ferric chloride (0.1% w/v) and the absorbance was read at 700 nm using ascorbic acid and gallic acid as standards. A higher absorbance of the reaction mixture indicates greater reducing power. The IC_{50} value ($\mu\text{g/mL}$) is the effective concentration of the extract at which the absorbance was 0.5 and it was obtained from linear regression analysis. All extracts were analysed in triplicate.

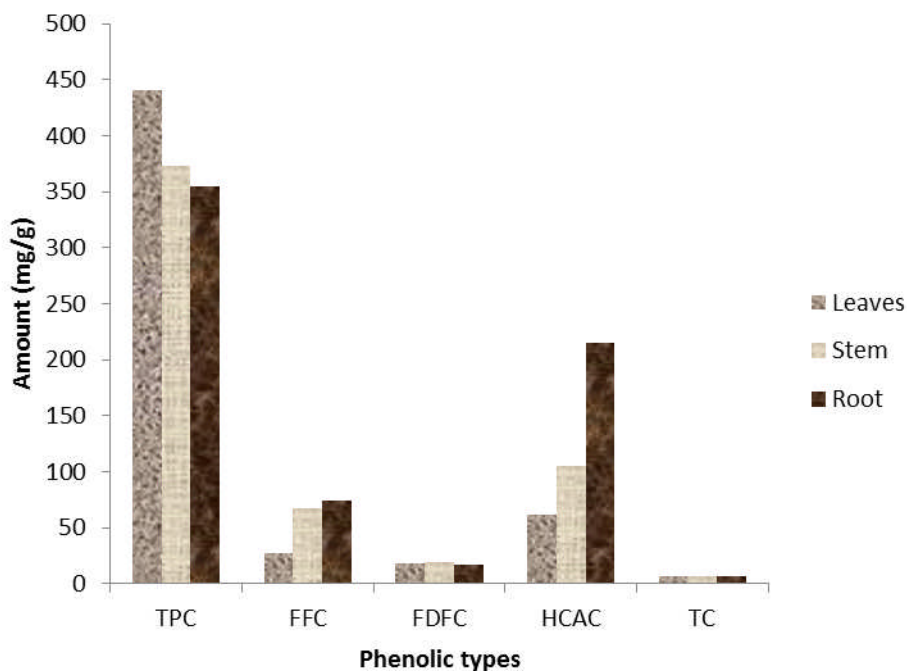
Results**Distribution of Total Phenols**

The total phenolic content of the extracts of different parts of *T. sericea* was determined using Folin-Ciocalteu reagent. The result (Table 1) shows that the hot water extract of the leaves had the highest phenolic content while the methanol/acetone extract of the root had the lowest content. In all plant parts, the water extracts (cold and hot) had higher phenolic content compared to the methanol/acetone extract. The average TPC (Figure 1) of the extracts showed that the leaves > stem > root.

Table 1: Distribution of polyphenols in the parts of *T. sericea*

	TPC	FFC	FDFC	HCAC	TC
Leaves					
Cold water	568.08±6.09 ^a	15.98±0.20 ^a	18.04±0.07 ^a	47.02±0.12 ^a	6.31±0.06 ^a
Hot water	649.52±0.55 ^b	25.22±0.74 ^b	26.44±0.08 ^b	64.33±0.29 ^b	13.20±0.04 ^b
Methanol/Acetone	103.02±2.30 ^c	39.62±1.69 ^c	9.76±0.03 ^c	73.26±1.23 ^c	1.90±0.03 ^c
Stem					
Cold water	535.69±6.67 ^d	65.62±1.16 ^d	28.36±0.03 ^d	81.74±0.78 ^d	1.99±0.02 ^c
Hot water	513.89±1.49 ^e	56.52±2.86 ^e	20.35±0.03 ^e	95.25±0.22 ^e	15.33±0.02 ^d
Methanol/Acetone	69.93±0.94 ^f	78.46±0.07 ^f	8.98±0.03 ^f	137.16±0.57 ^f	2.38±0.12 ^e
Root					
Cold water	500.83±4.97 ^g	67.37±0.22 ^d	18.00±0.04 ^a	184.75±0.42 ^g	2.57±0.02 ^f
Hot water	495.91±3.83 ^h	78.46±0.07 ^f	22.93±0.03 ^g	145.21±0.34 ^h	16.06±0.04 ^g
Methanol/Acetone	68.32±1.20 ^f	78.46±0.07 ^f	9.28±0.02 ^h	313.76±3.34 ⁱ	1.98±0.02 ^e

TPC: Total phenolic content (mgGAE/g), FFC: Flavone/flavonol content (mgQE/g), FDFC: Flavanones/dihydroflavonol content (mgQE/g), HCAC: Hydroxycinnamic acid derivatives content (mgCAE/g), TC: Tannin content (mgQE/g). Data are means of three replicates ± S.E. Values in the same column followed by the same letter are not significantly different ($p < 0.05$).

**Figure 1:** Average phenolic contents in organs of *T. sericea*

Distribution of Flavonoids

Our study showed (Table 1) that the concentration of flavonone/dihydroflavonol content (FDFC) in all the organs was lower than that of flavonol/flavone content (FFC). Methanol/acetone extract of the stem, hot water and methanol/acetone extracts of the roots had the highest flavonol/flavone content. Furthermore, the cold water extract of the stem had the highest flavonone/dihydroflavonol content. Methanol/acetone extracted more FFC from the leaves and stem compared to the water extracts. The hot water and methanol/acetone extracts of the root had the same FFC content. However, water (cold and hot) extracts contained higher FDFC in all the plant parts compared to methanol/acetone. The average FFC (Figure 1) showed that root > stem > leaves while the average FDFC showed that stem > leaves > roots.

Distribution of Hydroxycinnamic Acid Derivatives

The methanol/acetone extract of the roots (Table 1) had the highest concentration of hydroxycinnamic acid derivatives (313.76 mg/ml) and was significantly different from the other extracts. The root extracts (methanol/acetone, cold and hot water extracts) showed higher content compared to other organs regardless of the solvent used. The average HCAC (Figure 1) showed that root > stem > leaves.

Distribution of Tannins

In this study, the root hot water extract had the highest tannin content and was significantly different from the other extracts. Furthermore, the hot water extract of all the organs had higher tannin content (TC) compared to the cold water and methanol/acetone extract. The average TC (Figure 1) showed that leaves > root > stem.

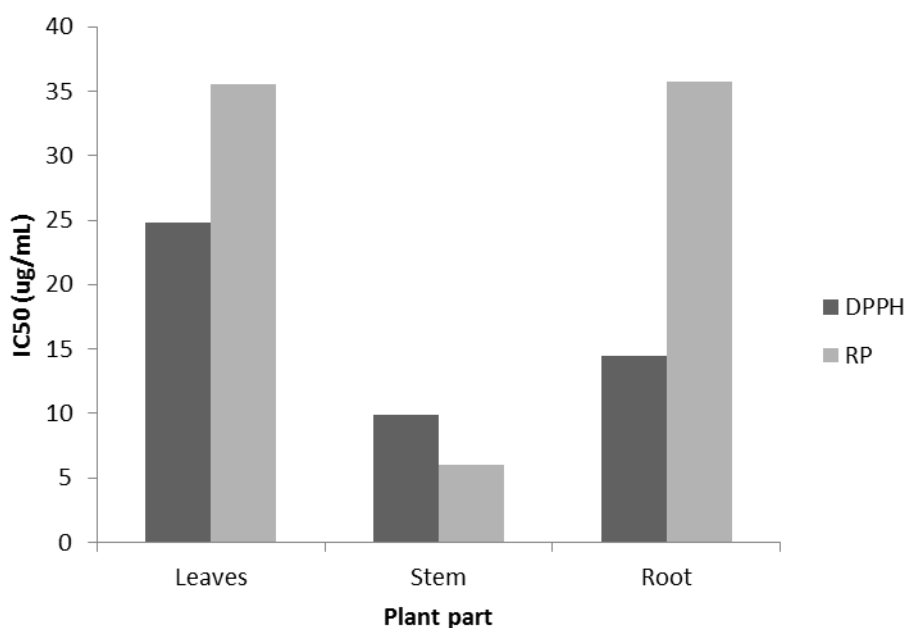
Free Radical Scavenging Activity (DPPH)

The hot water extract (HE) exhibited the highest free radical scavenging activity ($IC_{50} = 4.25 \mu\text{g/mL}$) in the leaves (Table 2); the methanol/acetone extract gave the highest free radical scavenging activity for the stem ($IC_{50} = 4.51 \mu\text{g/mL}$) and root ($IC_{50} = 2.4 \mu\text{g/mL}$) extracts, respectively. The stem extracts exhibited the lowest average IC_{50} value for free radical scavenging activity (Figure 2)

Table 2: Antioxidant activity ($IC_{50} \mu\text{g/ml}$) of parts of *T. sericea*

	Leaves	Stem	Root	Vit.C	Gallic Acid
DPPH					
CE	12.57±1.65 ^a	8.14±0.2 ^d	23.17±0.3 ^f	-	-
HE	4.25±0.49 ^b	16.91±0.17 ^e	17.89±0.76 ^g	-	-
MAE	57.68±1.56 ^c	4.51±0.62 ^b	2.4±0.06 ^b	4.63±0.15 ^b	3.04±0.14 ^b
RP					
CE	43.69±0.83 ^a	9.08±0.03 ^d	47.53±0.21 ^f	-	-
HE	2.95±0.22 ^b	0.31±0.03 ^e	0.86±0.02 ^e	-	-
MAE	59.88±1.27 ^c	8.63±0.06 ^d	58.71±0.74 ^c	76.03±0.64 ^g	49.72±1.91 ^f

Data are mean of three triplicates. Values in rows and columns of each antioxidant activity with the same alphabet are not significantly different ($P < 0.05$). CE: Cold water extract; HE: Hot water extract; MAE: Methanol/Acetone extract. Vit.C: Vitamin C.

**Figure 2:** Average antioxidant activity of organs of *T. sericea*

Reducing Power Activity

The result in table 2 shows that the hot water extracts (HE) had the highest reducing power for all the organs studied; however, there was no statistically meaningful difference between the reducing power activity of the extracts from stem and root. The stem extracts exhibited the lowest average IC₅₀ value for reducing power activity (Figure 2).

Pearson Correlation Analysis

The correlation analysis (Table 3) of all the organs showed that there was a significant ($P < 0.01$) relationship between TPC and FDFC and between FFC and HCA ($P < 0.05$). All the phenolics showed a weak negative correlation with DPPH while TC showed a significant negative correlation ($P < 0.05$) with RP. There was a positive correlation ($r = 0.477$) between DPPH and RP.

Table 3: Pearson correlation coefficients among compounds and antioxidant activity of *T. sericea* leaves, stem and root.

	FFC	FDFC	HCA	TC	DPPH	RP
TPC	-0.437	0.903**	-0.514	0.583	-0.209	-0.477
FFC		-0.223	0.699*	0.160	-0.278	-0.142
FDFC			-0.464	0.523	-0.290	-0.628
HCAC				-0.285	-0.312	0.378
TC					-0.154	-0.668*
DPPH						0.477

** Correlation is significant at $P < 0.01$ level.

* Correlation is significant at $P < 0.05$ level.

Discussion

Distribution of Total Phenols

The extracts of the aerial parts (leaves and stem) showed higher phenolic content than the root and this is similar to the study of Falleh et al. (2011). However, the study of Falleh et al. (2011) reported that the stem had the highest phenolic content. According to Falleh et al. (2008), the solubility of phenolic compounds is governed by the type of solvent used for extraction, the degree of polymerization and the interaction of phenolics with other food constituents and formation of insoluble complexes. The ability of solvents to extract polyphenols is greatly influenced by their polarity and the amount of phenolic compounds may increase with an increase in solvent polarity (Falleh et al., 2013). The high phenolic content in the water extracts of the leaves could be as a result of interferences such as sugar, ascorbate and aromatic acids which are associated with the use of Folin-Ciocalteu reagent (Boulanouar et al., 2013) and are more soluble in water than organic solvents. The high phenolic content of the hot water extract of the leaves suggests that more phenolics are extracted by decoction of the leaves compared to infusion. Since most traditional healers use water for their preparations (Kaneria et al., 2012), it could be deduced that high quantities of phenolics are extracted and this could be linked to the therapeutic effect of the concoctions. In our study, the leaves had the highest phenolic content compared to the extracts of the stem and root.

Distribution of Flavonoids

Flavones and flavonols are known as anthoxanthins and are the most abundant group of flavonoids and are primarily responsible for the colour of many flowers ranging from yellow to white. Flavonones and dihydroflavonols are also widely distributed among higher plants but are found in low concentrations. They are fungitoxic compounds useful in wood preservation (Daniel, 2006). The result of the flavonoid contents suggest that methanol/acetone was a better solvent for extracting FFC while water was a better extracting solvent for FDFC. In other words, FFC are likely to be a mixture of both polar and non-polar compounds while FDFC may constitute more polar compounds. The presence of flavonoids in *T. sericea* may function as anti-herbivore (prooxidant activity) in addition to its UV-screening function (antioxidant activity). The root possessed the highest FFC while the stem possessed the highest FDFC.

Distribution of Hydroxycinnamic Acid Derivatives

Our study shows that the leaves contained the highest phenolic content; however, the root contained the highest hydroxycinnamic acid derivative content (HCAC). This suggests that phenolics other than hydroxycinnamic acid derivatives could be responsible for the high content in the leaves. It was also observed that the methanol/acetone extracts of all plant parts had higher hydroxycinnamic acid derivative contents than the cold and hot water extracts.

Distribution of Tannins

Tannins are plant polyphenols that are generally classified as hydrolysable and condensed. The former group is soluble in water while the latter is insoluble in water. The hydrolysable tannins are abundant in leaves while the condensed tannins are concentrated in the wood (Daniel, 2006). This study has shown that the amount of condensed tannins (also known as proanthocyanidins) varied among the organs with the hot water extract of the root exhibiting the highest concentration. Our study is in agreement with Tuominen et al. (2013b) who reported that hydrolysable tannins were predominant in the leaves of *Geranium sylvaticum* while the roots predominantly contained proanthocyanidins (PA).

Identification of the polyphenols in *T. sericea* will help to understand how and why they are distributed in the different plant parts.

Free Radical Scavenging Activity (DPPH)

DPPH is used to access the free radical scavenging activity of compounds. The ability of compounds or medicinal plants to donate hydrogen to radicals increases their ability to scavenge such radicals (Ye et al., 2013). In *T. sericea*, the free radical scavenging compounds in the leaves are likely to be very polar compounds while the free radical scavenging compounds found in the stem and root may be partly due to highly polar and less polar compounds. The result of the free radical scavenging activity of the extracts from leaves in this study agrees with the study of Teixeira et al. (2012), who reported that the hot water extract of *Mentha pulegium* exhibited the highest free radical scavenging activity and phenolic content compared to the cold water extract, ethanol extract and essential oil.

Reducing Power Activity

The reducing power of an antioxidant is measured by its ability to donate electrons to free radicals, converting them to more stable products and finally terminating the radical chain reaction (Cao et al., 2009). The result of the reducing power activity showed that compounds with reducing power activity in *T. sericea* are best extracted with hot water regardless of the organ. This is in agreement with the report of Teixeira et al. (2012) who reported that hot water exhibited the highest antioxidant activity in *Mentha pulegium*. Comparing the reducing power of the plant with its free radical scavenging activity, it is obvious that the antioxidant activity of the leaves is best extracted with water. While methanol/acetone extracted more DPPH free radical scavengers in the stem and root, hot water extracted more reducing power compounds. This showed that *T. sericea* exerts its antioxidant activity through proton and electron donations and these compounds could be polar and non-polar. According to Ye et al. (2013), it is not sufficient to use one antioxidant test to establish the antioxidant activity of medicinal plant since the plants have complex phytochemical facets. At least two tests should be used to establish authenticity. The average antioxidant activity (Figure 2) showed that the stem exhibited the highest free radical scavenging and reducing power activities compared to the leaves and root.

Pearson Correlation Analysis

From the result in table 1, it was observed that the water extracts gave the highest TPC and FDFC while methanol/acetone extract gave the highest FFC and HCAC. This suggests that water is a better extracting solvent for TPC and FDFC while polar organic solvent (in this case methanol/acetone) is a better extracting solvent for FFC and HCAC. In practical terms, it could mean that herbal preparations with alcoholic beverages may extract more FFC and HCAC compared to water. Looking at the plant as a whole, the electron donating capacity was more pronounced compared to its hydrogen donating capacity. Furthermore, tannins could be responsible for the antioxidant activity of the plant. The relationship between DPPH and RP showed that about 47% of the compounds responsible for the free radical scavenging activity also possess reducing power activity.

Conclusion

This study has evaluated the distribution of TPC, HCAC, FFC, FDFC and TC in the leaves, stem and root of *T. sericea*. TPC and TC were found in higher amounts in the leaves, FDFC in the stem while HCAC and FFC had the highest contents in the root. Considering the choice of extracting solvent, water is better for extracting phenolic acids (TPC) and Flavanones/dihydroflavonol compounds while polar organic solvent is better for extracting Flavone/flavonol and Hydroxycinnamic acid derivative compounds. Furthermore, the stem extracts exhibited higher antioxidant activity compared to the extracts from leaves and root. The study also showed that tannins were responsible for the reducing power activity. Confirmatory studies are ongoing to determine the actual distribution of phenols and their antioxidant capacity in *T. sericea*.

The authors hereby declare no conflict of interest

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